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Rapid Detection of *Campylobacter coli*, *C. jejuni*, and *Salmonella enterica* on Poultry Carcasses by Using PCR–Enzyme-Linked Immunosorbent Assay

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Contamination of retail poultry by *Campylobacter* spp. and *Salmonella enterica* is a significant source of human diarrheal disease. Isolation and identification of these microorganisms require a series of biochemical and serological tests. In this study, *Campylobacter ceuE* and *Salmonella invA* genes were used to design probes in PCR–enzyme-linked immunosorbent assay (ELISA), as an alternative to conventional bacteriological methodology, for the rapid detection of *Campylobacter jejuni*, *Campylobacter coli*, and *S. enterica* from poultry samples. With PCR-ELISA (40 cycles), the detection limits for *Salmonella* and *Campylobacter* were 2×10^2 and 4×10^1 CFU/ml, respectively. ELISA increased the sensitivity of the conventional PCR method by 100- to 1,000-fold. DNA was extracted from carcass rinses and tetrathionate enrichments and used in PCR-ELISA for the detection of *Campylobacter* and *S. enterica*, respectively. With PCR-ELISA, *Salmonella* was detected in 20 of 120 (17%) chicken carcass rinses examined, without the inclusion of an enrichment step. Significant correlation was observed between PCR-ELISA and cultural methods (kappa = 0.83; chi-square test, $P < 0.001$) with only one false negative (1.67%) and four false positives (6.67%) when PCR-ELISA was used to screen 60 tetrathionate enrichment cultures for *Salmonella*. With PCR-ELISA, we observed a positive correlation between the ELISA absorbance (optical density at 405 nm) and the campylobacter cell number in carcass rinse, as determined by standard culture methods. Overall, PCR-ELISA is a rapid and cost-effective approach for the detection and enumeration of *Salmonella* and *Campylobacter* bacteria on poultry.

An estimated 76 million cases of food-borne illnesses occur annually in the United States, of which 5,200 are fatal (37). *Campylobacter* and *Salmonella* bacteria account for 2.4 million and 1.4 million of these cases, respectively. Poultry and poultry products have been implicated as a major source of *Campylobacter* and *Salmonella* infection in humans (5, 15). *Salmonella* bacteria generally cause a self-limiting gastroenteritis in healthy adults and occasionally cause a sometimes-fatal bacteremia in the very young or the elderly (62). For *Campylobacter* species, most human infections are caused by *C. jejuni* and *C. coli* (3). Although rarely fatal, *C. jejuni* infection can sometimes cause a debilitating neurological disorder, Guillain-Barré syndrome (11). A recent study revealed that 70.7% of the poultry carcasses and 91% of the retail chicken products examined were contaminated with *Campylobacter* (63). Based on U.S. Department of Agriculture (USDA)-Food Safety and Inspection Service surveillance, the prevalence of *Salmonella* contamination of freshly processed poultry carcasses was reported to be 11.4% in 1999 and 9.1% in 2000 (http://www.usda.gov). Hazard analysis and critical control point systems for poultry are being implemented currently in the United States and other countries (39). Beginning in 1996, large poultry processors have been required to meet performance standards for reducing the frequency of *Salmonella* contamination (2). These events and the short shelf life of meat products have stimulated the development and implementation of rapid and specific detection methods for those pathogens in poultry.

Conventional cultural methods for detecting *Salmonella* and *Campylobacter* spp. involve enrichment in selective broth, followed by isolation on selective differential agar. *Campylobacter* spp. have demanding growth requirements because they need to be incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), which makes the task of isolation laborious and costly (20). Both a primary and a secondary enrichment culture are necessary for isolating *Salmonella enterica* from foods. Isolation can therefore be labor-intensive and expensive when large numbers of samples must be processed (34). Hence, there is need for a sensitive noncultural detection method for these food-borne pathogens.

Molecular techniques such as PCR have proven to be specific and sensitive methods for detecting infectious pathogens (7, 29). PCR can detect as few as 100 bacteria per milliliter (18). Direct identification of organisms without prior isolation and purification from samples such as urine, sputum, poultry carcass rinses, and fecal material can be done (30, 50, 57, 58). The PCR–enzyme-linked immunosorbent assay (ELISA) has also been used to detect *Campylobacter* and *S. enterica* in environmental water, stools, and poultry samples and is more

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sensitive than conventional gel-based PCR. PCR-ELISA involves incorporation of chemically tagged nucleotides into the PCR amplicon and subsequent detection of the PCR product with antibody-enzyme conjugate that recognizes the unique chemical label present in the incorporated nucleotides (33). Under most circumstances, samples require an enrichment step or must be concentrated to improve the likelihood of detecting the target organisms by PCR (24, 35, 46, 48). In this study, we designed, validated, and implemented a specific, multiplex PCR primer set and probes based on the *Salmonella* virulence gene *invA* (12) and the *Campylobacter ceuE* gene, which encodes a lipoprotein involved in siderophore transport (42), for PCR-ELISA to screen poultry carcasses for these two important food-borne pathogens.

TABLE 1. PCR primers and ELISA capture probes for *S. enterica*, *C. coli*, and *C. jejuni*

Target gene	Nucleotide sequence (5′→3′)	Position[a]	Expected product size (bp)
invA (*Salmonella*)			
*invA*F	F: CGCTCTTTCGTCTGGCATTATC	224–245	408
*invA*R	R: CCGCCAATAAAGTTCACAAAG	632–612	
invA capture probe	TTTCTCTGGATGGTATGCCC	428–447	
ceuE (*Campylobacter*)			
JCF1	F: TTAGTATGAGCGATGAGGGTG	137–157	610
JCR2	R: CTTTTTCCGTGTGTGCCTAC	749–730	
ceuE capture probe	ATCATTTCTGGACGCCAAAG	400–419	

[a] Positions of the forward and reverse primers in the target gene sequence.

MATERIALS AND METHODS

Isolation of *Salmonella* and *Campylobacter* from poultry and the poultry environment. For the *Campylobacter* study, 32 chicken carcasses were removed from shackles in a commercial processing plant. Sixteen were collected prior to the inside-outside washer, and 16 were collected immediately following the inside-outside washer. Each carcass was individually placed into a sterile plastic bag, sealed, covered with ice, and transported to the laboratory. Sterile distilled water (100 ml) was used to rinse the carcass, and the rinse was cultured for *Campylobacter* (19). The carcass rinse was serially diluted (1:10) in phosphate-buffered saline (PBS), and *Campylobacter* bacteria were enumerated by plating the mixture in duplicate onto Campy-Cefex agar (54). One-tenth milliliter was spread onto each plate with a sterile plastic inoculating loop, and plates were subsequently incubated at 42°C for 36 to 48 h in a microaerobic environment (5% O_2, 10% CO_2, and 85% N_2). Colonies characteristic of *Campylobacter* were counted. Each colony type counted as *Campylobacter* from each sample was confirmed as a member of the genus by microscopic examination for typical helical cellular morphology and detection of darting motility. Each colony was identified as *C. jejuni* or *C. coli* with a *Campylobacter* species-specific latex agglutination test kit (Integrated Diagnostics Inc., Baltimore, Md.) (8).

For detection of *S. enterica*, commercial broiler houses were sampled by using drag swabs, which were gauze pads soaked with double-strength skim milk (13). Each drag swab was then placed in 100 ml of tetrathionate brilliant green broth (TBG) (Becton Dickinson and Co., Sparks, Md.) and incubated at 41.5°C for 18 h (10). Chicken carcasses collected at the processing plant prior to and immediately after chilling were rinsed with 250 ml of buffered peptone water (59). Ten milliliters of the carcass rinse was used to inoculate 90 ml of TBG enrichment broth, and the enrichment broth was incubated at 41°C for 18 h (10). A loopful of the enrichment broth was streaked onto an XLT4-BGN biplate (Becton Dickinson and Co.) followed by overnight incubation at 37°C (25). H_2S-producing colonies on XLT4 plates were identified as *Salmonella* by using poly(O) *Salmonella*-specific antiserum (Becton Dickinson and Co.) in a whole-cell agglutination assay.

PCR-ELISA for detecting *C. jejuni*, *C. coli*, and *S. enterica*. DNA was extracted from TBG enrichment broth of the drag swabs as described by Liu et al. (34). The Mo Bio DNA purification and isolation kit (Mo Bio Laboratories Inc., Solana Beach, Calif.) was used to extract DNA from *Campylobacter* and *Salmonella* in chicken carcass rinses. Carcass rinse aliquots (50 ml) were held at 4°C for 30 min, and fat was then separated by centrifugation at 820 × *g* for 15 min. The supernatant containing bacteria was transferred to a second tube, and cells were sedimented by centrifugation at 19,000 × *g* for 15 min. The resulting bacterial pellet was resuspended in 1 ml of PBS (pH 7.0) and transferred to 2 ml of Bead Solution tubes. DNA was isolated and purified according to the protocol described by the manufacturer and was then used directly in PCR. The final volume of DNA eluted from the DNA-affinity column was 30 μl. PCR was done to evaluate the quality of the templates by using universal 16S rRNA primers and confirming the expected size amplicon (995 bp) on an agarose gel (43). DNA was separated on a 1.6% agarose–1× Tris-acetate-EDTA (pH 8.0) gel with ethidium bromide (0.2 μg/ml) at 100 V (49). A 100-bp ladder (Roche Molecular Biochemicals, Indianapolis, Ind.) was used as a molecular size standard for determining the size of PCR products.

Campylobacter ceuE, a gene encoding a 34.5- to 36.2-kDa lipoprotein component of a binding-protein-dependent transport system for the siderophore enterochelin, was analyzed with GeneRunner (Hastings Software, Hastings, N.Y.) DNA analysis software to search for PCR primers specific for *C. jejuni* (GenBank accession no. NC 002163) and *C. coli* (GenBank accession no. X88849) (42) (Table 1). *Salmonella* virulence gene *invA* was used to design PCR primers specific for *S. enterica* (GenBank accession no. M90846) (12) (Table 1). The capture probe was also designed to anneal to the central region of the PCR amplicon. A biotin molecule was added to the 3′ end of the probe to prevent it from serving as a primer in the PCR, and this oligonucleotide was used to bind the PCR amplicon to the bottom of the ELISA plate coated with streptavidin. Primers and probes were synthesized at the Molecular Genetics Instrumentation Facility at the University of Georgia with the ABI Model 394 oligonucleotide synthesizer. PCR was done with a Rapidcycler hot-air thermocycler (Idaho Technologies, Idaho Falls, Idaho) with 10-μl-capacity capillary tubes (61). The 10-μl PCR mix consisted of 3.0 mM $MgCl_2$, 50 mM Tris (pH 8.3), 0.25 mg of bovine serum albumin/ml, 1.0 μM (each) primer, 2.0 pM biotin-labeled *Campylobacter* or *Salmonella* probe, 0.2 mM digoxigenin (DIG)-labeled deoxynucleotides, 1.0 U of *Taq* DNA polymerase, and 1 μl of DNA template. The PCR program parameter consisted of a hold at 94°C for 1 min; then 94°C for 0 s, 55°C for 0 s, and 72°C for 20 s with a ramping rate of 2.0°C/s between the annealing and extension steps for 25, 30, or 40 cycles; and a final extension at 72°C for 4 min. An additional cycle was added to the final stage of the PCR amplification to anneal the detection probe to PCR amplicons. For *Campylobacter*, this additional cycle step consisted of 94°C for 1 min and 42°C for 16 min with a ramping rate of 2.0°C/s between the annealing and extension steps, whereas the PCR program for *Salmonella* had a final step of 94°C for 1 min and 50°C for 16 min with a ramping rate of 2.0°C/s between the annealing and extension steps. PCR amplicons were detected with a DIG detection ELISA kit (ABTS [2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid)]; Roche Molecular Biochemicals, Mannheim, Germany). PCR product was placed in streptavidin-coated wells containing conjugate buffer (PBS [pH 7.0] plus 0.1% blocking reagent) and incubated at 37°C for 1 h. The wells were then washed five times with wash buffer (PBS plus 0.1% Tween 20), with the buffer being left in wells for 2 min with each wash. Anti-DIG antibody–peroxidase conjugate (at the dilution specified by the manufacturer) was added to each well and incubated at 37°C for 1 h before the wells were washed five times with the wash buffer. Finally, the peroxidase substrate was added to wells and optical density (OD) values were recorded with an ELISA plate reader (Molecular Devices Corp., Sunnyvale, Calif.) (λ = 405 nm) after 1 h of incubation at room temperature.

To determine the detection limit for *Campylobacter*, genomic DNA was extracted with the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, Minn.) from *C. jejuni*. DNA was quantified with the GeneQuant II RNA-DNA calculator (Amersham Biosciences Corp., Piscataway, N.J.). Tenfold serial dilutions were made from the extracted DNA (34.6 μg/μl) and tested with PCR-ELISA. Based on the amount of DNA in each bacterial cell (8.8×10^{-15} g), the detection limit was converted from the unit micrograms per microliter to CFU per milliliter (27). To determine the detection limit for *S. enterica*, we first cultured the bacteria overnight in Luria-Bertani medium and DNA was extracted according to the cetyltrimethylammonium bromide protocol of Ausubel et al. (4). Tenfold serial dilutions were made from extracted DNA. PCR-ELISA was then performed as described above. Specificity was determined for PCR-ELISA for *S. enterica* serotypes Typhimurium ($n = 4$), Enteritidis ($n = 3$), Hadar ($n = 2$), Heidelberg ($n = 2$), Kentucky ($n = 2$), Agona ($n = 1$), Anatum ($n = 1$), Bredney ($n = 1$), Chester ($n = 1$), Choleraesuis ($n = 1$), Infantis ($n = 1$), Indiana ($n =$

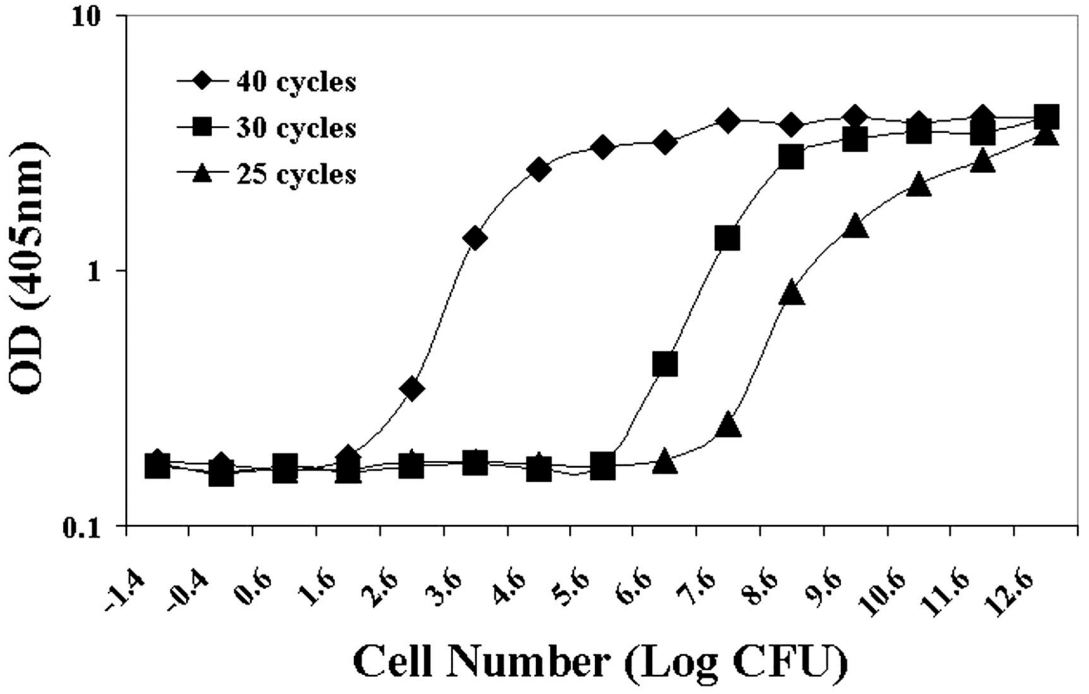

FIG. 1. Correlation between *Campylobacter* PCR-ELISA OD_{405}s and *Campylobacter* cell count and the sensitivity of PCR in detecting *C. coli* or *C. jejuni* as a function of the number of PCR cycles. Arrows point to the reflection points (log CFU per milliliter) where the log-linear phase of OD readings begins for different cycles of PCR amplification.

1), Give ($n = 1$), London ($n = 1$), Montevideo ($n = 1$), Mbandaka ($n = 1$), Muenchen ($n = 1$), Ohio ($n = 1$), Schwarzengrund ($n = 1$), and St. Paul ($n = 1$) and for other *Enterobacteriaceae* including *Citrobacter freundii* ($n = 1$), *Klebsiella pneumoniae* ($n = 1$), *Serratia fonticola* ($n = 1$), *Enterobacter cloacae* ($n = 1$), and *Escherichia coli* ($n = 10$). Specificity for *Campylobacter* was determined with *C. jejuni* ($n = 12$), *C. coli* ($n = 6$), *C. lari* ($n = 2$), *C. upsaliensis* ($n = 1$), and *C. fetus* ATCC 25936 ($n = 1$), and the other ε-proteobacteria including *Helicobacter hepaticus* ($n = 1$), *Arcobacter cryaerophilus* ATCC 49942 ($n = 1$), and *Arcobacter butzleri* ATCC 49616 ($n = 1$). *C. jejuni* and *C. coli* strains were isolated from poultry litter samples. *C. lari* and *C. upsaliensis* strains were isolated from canine feces.

C. lari, *C. upsaliensis*, *C. fetus*, *H. hepaticus*, *A. cryaerophilus*, and *A. butzleri* were cultured on blood agar in a microaerobic atmosphere containing approximately 5% O_2, 10% CO_2, and 85% N_2, generated with the Pack-Campylo Anaeropack system (Mitsubishi Gas Chemical Company, Inc., New York, N.Y.), at 37°C for 36 h. Bacterial colonies were scraped off the plates, and the DNA template for PCR was prepared by the boiling method (36).

Statistics. The kappa test and chi-square test were performed to evaluate the correlation between cultural and PCR-ELISA methods for detecting *S. enterica*, *C. jejuni*, and *C. coli*. Sensitivity and specificity were also calculated from the contingency table generated for the kappa and chi-square tests. StatXact-3 version 3.0 (Cytel Software Corporation, Cambridge, Mass.) was used to perform statistical analysis.

RESULTS

PCR-ELISA to detect *Campylobacter* sp., *C. jejuni*, and *C. coli* on chicken carcasses. We determined the specificity of the PCR-ELISA for *C. jejuni* ($n = 12$), *C. coli* ($n = 6$), *C. lari* ($n = 2$), *C. upsaliensis* ($n = 1$), *C. fetus* ($n = 1$), *E. coli* ($n = 6$), *Salmonella* ($n = 5$), *H. hepaticus* ($n = 1$), *A. cryaerophilus* ($n = 1$), *A. butzleri* ($n = 1$), *C. freundii* ($n = 1$), *K. pneumoniae* ($n = 1$), *S. fonticola* ($n = 1$), and *E. cloacae* ($n = 1$). The PCR-ELISA values for OD at 405 nm (OD_{405}) for the negative control strains (non-*C. jejuni*-*C. coli*) (OD_{405} range, 0.162 to 0.235) were recorded and served as the cutoff point for identifying positives. They were the mean plus 2 (cutoff point, 0.22) or 4 (cutoff point, 0.26) standard deviations. Any reaction with its OD_{405} between 0.22 and 0.26 was judged as weakly positive while those with OD_{405}s greater than 0.26 were considered strongly positive. The OD_{405} range for *C. jejuni* ($n = 12$) and *C. coli* ($n = 6$) was between 0.26 and 4.0.

In developing a PCR-ELISA, we envisioned a test that could detect the target pathogen directly from the sample, without a preenrichment step. It was therefore necessary to optimize the PCR to detect the fewest number of cells possible per reaction. One PCR parameter that greatly influences sensitivity is cycle number; increasing the number of cycles increases the sensitivity of the PCR (45). PCR-ELISA was conducted on 10-fold serial dilutions of DNA template with a PCR program of 25, 30, and 40 cycles. Studies were done in triplicate. OD_{405}s were recorded, averaged, and plotted against cell number (Fig. 1). For 40 PCR cycles, the reflection point (OD versus cell density) was 4.0×10^2 CFU/ml, while with 30 and 25 cycles of PCR amplification, the reflection points were 4.0×10^6 and 4.0×10^7 CFU/ml, respectively. The detection limit of PCR-ELISA on *Campylobacter* sp. was determined to be as low as 346 fg, or the equivalent of 40 CFU/ml for 40 PCR cycles. There was a linear correlation ($R = 0.987$; $P < 0.001$) between OD_{405} and cell density for 40 PCR cycles with the minimum limit of detection at 40 CFU/ml. For cell densities of $>10^6$ CFU/ml, it was necessary to dilute the sample 10-fold in order for the signal to fall within the linear detection range of PCR-ELISA and to estimate campylobacter cell numbers by this method.

Next, we applied our PCR-ELISA toward the detection and enumeration of *C. coli* and *C. jejuni* directly from carcass rinse. Thirty-two chicken carcass rinses were cultured under mi-

croaerobic conditions for detection of *Campylobacter* spp. DNA was also extracted from the same carcass rinses for PCR-ELISA. All the samples were *Campylobacter* positive based on the culturing method, with the highest *Campylobacter* cell count at 3.16×10^4 CFU/ml and the lowest *Campylobacter* cell count at 5 CFU/ml. With PCR-ELISA, 31 of 32 samples were PCR positive for *Campylobacter*. Among them, there were 4 weak positives (OD_{405}, 0.22 to 0.255) and 27 strong positives (OD_{405}, 0.28 to 2.2). There was one false negative (3.1%). There was a linear correlation between PCR-ELISA OD and colony counts ($R = 0.792$; $P < 0.001$) (Fig. 2A).

PCR-ELISA to detect *S. enterica* present in poultry farm drag swabs and chicken carcass rinses. To determine the specificity of the probes, we performed PCR-ELISA on *S. enterica* serotypes Typhimurium ($n = 4$), Enteritidis ($n = 3$), Hadar ($n = 2$), Heidelberg ($n = 2$), Kentucky ($n = 2$), Agona ($n = 1$), Anatum ($n = 1$), Bredney ($n = 1$), Chester ($n = 1$), Choleraesuis ($n = 1$), Infantis ($n = 1$), Indiana ($n = 1$), Give ($n = 1$), London ($n = 1$), Montevideo ($n = 1$), Mbandaka ($n = 1$), Muenchen ($n = 1$), Ohio ($n = 1$), Schwarzengrund ($n = 1$), and St-Paul ($n = 1$) and on other members of the *Enterobacteriaceae*, including *Citrobacter freundii* ($n = 1$), *Klebsiella pneumoniae* ($n = 1$), *Serratia fonticola* ($n = 1$), *Enterobacter cloacae* ($n = 1$), and *Escherichia coli* ($n = 10$). PCR-ELISA OD_{405}s for negative control strains (non-*Salmonella*) were recorded (OD_{405} range, 0.205 to 0.418) and served as the cutoff point for identifying positives. They were the mean plus 2 (cutoff point, 0.48) or 4 (cutoff point, 0.67) standard deviations. Any reaction with an OD_{405} above 0.48 was considered weakly positive, and any with an OD_{405} above 0.67 was recorded as strongly positive. Of the 20 different *S. enterica* serotypes examined, all were positive by PCR-ELISA with OD values of ≥ 0.67. The OD_{405} range for *Salmonella* ($n = 20$) was 0.67 to 4.0. The detection limit of PCR-ELISA was found to be 2×10^2 CFU/ml for 40 PCR cycles. There was also a linear correlation ($R = 0.924$; $P < 0.001$) between OD_{405} and cell density (Fig. 2B).

This molecular biology-based method was first applied as a screen for *Salmonella* in overnight TBG enrichments ($n = 60$) of drag swabs. Thirty samples were culture positive for *Salmonella*, while PCR-ELISA identified 33 samples positive for *Salmonella* (OD_{405} range, 0.528 to 3.983) (Table 2). Of the samples positive for *Salmonella* by only one method, one was culture positive but PCR-ELISA negative and four were PCR-ELISA positive but culture negative (relative sensitivity, 0.97; relative specificity, 0.87). There was excellent correlation between the two methods as evidenced by the kappa test ($\kappa = 0.83$) and the chi-square test ($P < 0.001$).

We then directly screened 120 chicken carcass rinses for *Salmonella* by PCR-ELISA, of which 30 samples were culture positive. The PCR-ELISA identified 26 positive samples directly from carcass rinses, without an overnight preenrichment step. Seventeen of these samples yielded strongly positive reactions, with their OD_{405} readings being >0.67. However, some discrepancies were found between the culture and PCR-ELISA results. Ten samples were culture positive but ELISA negative. Another six samples were PCR-ELISA positive but culture negative. The PCR-ELISA for *Salmonella* yielded 5.0% false positives and 8.3% false negatives based on the assumption that the cultural method is the standard for detection (relative sensitivity, 0.67; relative specificity, 0.93) (Table 2). The chi-square test indicated a significant correlation between PCR-ELISA and culture methods for detecting *Salmonella* ($P < 0.001$), while there was good agreement according to the kappa test (0.63). Based on OD_{405}s (range, 0.489 to 2.657), the estimated level of *Salmonella* contamination was between 2×10^2 and 2×10^4 CFU/ml for the carcass rinse.

DISCUSSION

Conventional cultural methods for isolating *Campylobacter* and *Salmonella* are time-consuming and labor-intensive, especially when a large number of samples are tested. This study evaluated PCR-ELISA as an alternative to a cultural detection method for *C. jejuni*, *C. coli*, and *S. enterica*. The lipoprotein-encoding gene *ceuE* had been successfully used as the target for specific identification of certain *Campylobacter* species (23). However, the relative sensitivity and specificity for the identification of *C. jejuni* are 0.88 and 0.98, respectively, by use of these *ceu*-based PCR primers (40). We therefore designed our PCR primers and capture probes to a region of *ceuE* conserved between *Campylobacter* species *C. coli* and *C. jejuni* in order to reduce the chance of false negatives due to sequence divergence in *ceuE* among *C. coli* and *C. jejuni* isolates. The PCR-ELISA was specific to *C. coli* and *C. jejuni*. With our primers and capture probe, PCR-ELISA was also sensitive enough to detect campylobacters *C. coli* and *C. jejuni* in carcass rinse at levels as low as 40 CFU/ml. Salis et al. indicated that their PCR-ELISA could detect one *Campylobacter* cell per reaction but only with purified DNA template (46). PCR-ELISA has been applied toward the detection of *Campylobacter* species from poultry (41), clinical samples (32), and environmental waters (48), but few studies have evaluated the utility of PCR-ELISA for detecting *Campylobacter* directly from samples. Lawson et al. reported using PCR-ELISA to detect *Campylobacter* directly from fecal samples (32). Though their probes could identify other *Campylobacter* species, they obtained 12.9% false positives and 9.6% false negatives for *C. jejuni* and *C. coli*. The one false negative reported in this study was due to campylobacter cell density, at 5 CFU/ml, being below the minimum detection limit of the PCR-ELISA.

In this study, we were also able to detect *Salmonella* present in chicken carcass rinses and broth enrichments by PCR-ELISA. ELISA has been reported previously to increase both the sensitivity and the specificity of PCR over those of gel electrophoresis-based PCR assays (32, 41). PCR-ELISA avoids possible subjective interpretations in PCR due to "nonspecific products" or "bands of unknown origin" (60). To our knowledge, the only study to evaluate the utility of this technique in detecting *Salmonella* was conducted by Luk et al. (35). They developed PCR-ELISA to detect the amplified lipopolysaccharide *rfbS* gene as a means for rapid screening of serogroup D S*almonella* in stool specimens. The test is specific for group D *Salmonella* with a detection limit of 10 bacteria per reaction when pure bacterial cultures are used. PCR-ELISA in our study increased sensitivity by 1,000-fold (40 CFU/ml) for *Campylobacter* and 100-fold (2×10^2 CFU/ml) for *Salmonella* over that of gel-based PCR. This is in agreement with the studies of Lawson et al. and O'Sullivan et al. (32, 41).

The false negatives associated with *Salmonella*-specific PCR-ELISA were not due to divergence among the target sequence,

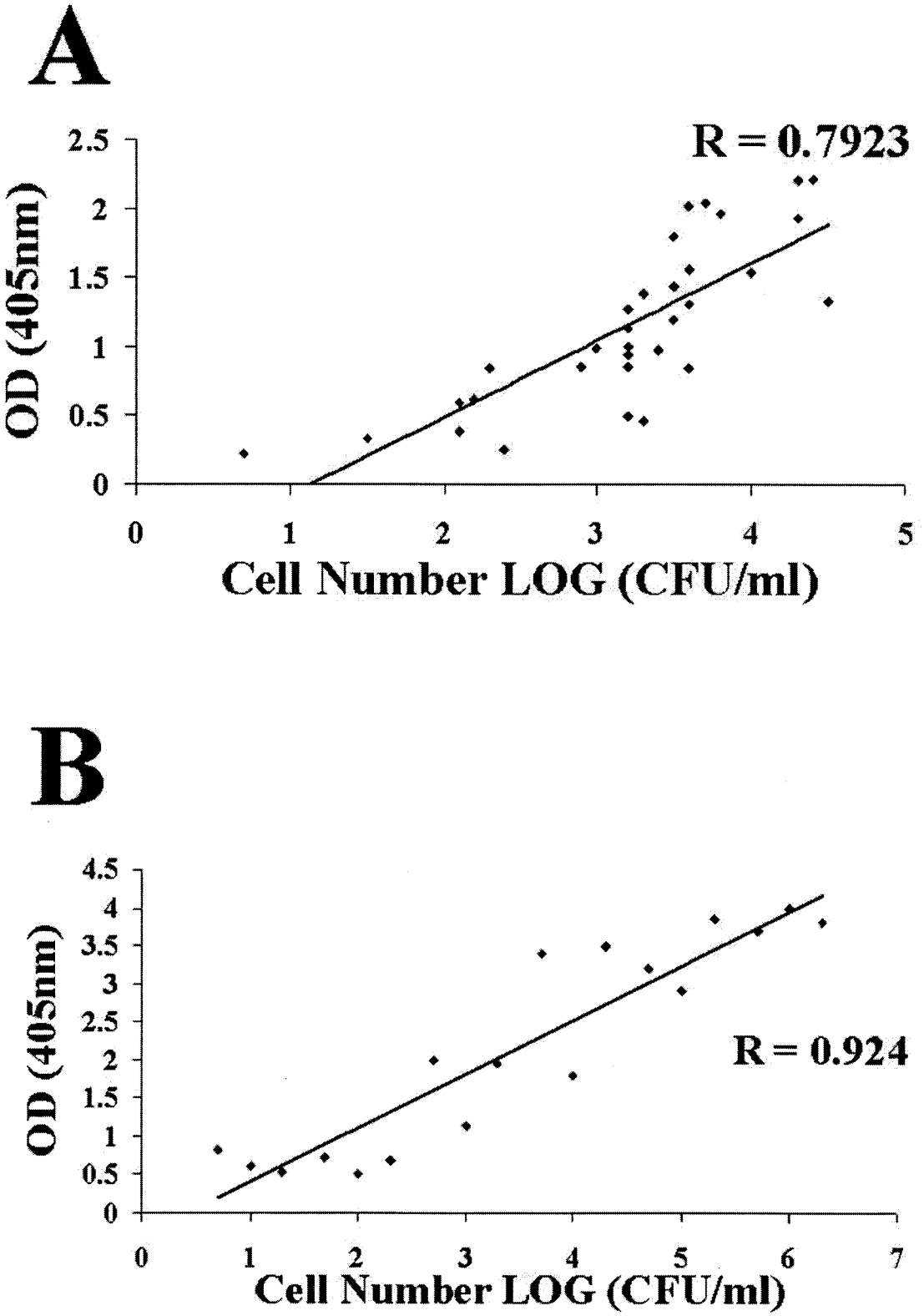

FIG. 2. Linear correlation between PCR-ELISA OD_{405}s and bacterial cell density as determined by traditional plate count. (A) PCR-ELISA OD_{405}s versus *C. coli* or *C. jejuni* cell concentration in chicken carcass rinses (Pearson correlation, $P < 0.001$). (B) PCR-ELISA OD_{405}s versus *Salmonella* cell concentration in samples (Pearson correlation, $P < 0.001$).

TABLE 2. Comparison of PCR-ELISA with culture method for detecting *S. enterica*, *C. coli*, and *C. jejuni* on poultry or in the poultry production environment

Sp. detected	No. of samples with result/total no.				Overall agreement[c]
	PCR-ELISA$^+$, culture$^+$	PCR-ELISA$^+$, culture$^-$	PCR-ELISA$^-$, culture$^+$	PCR-ELISA$^-$, culture$^-$	
Campylobacter[a]	31/32	0/32	1/32	0/32	31/32 (0.97)
Salmonella (direct)[a]	20/120	6/120	10/120	84/120	104/120 (0.87)
Salmonella (enrichment)[b]	29/60	4/60	1/60	26/60	55/60 (0.92)

[a] Detection of pathogen in chicken carcass rinses.
[b] Detection of *S. enterica* in samples following overnight enrichment culture in TBG.
[c] Number of true positives plus true negatives divided by the total sample number.

invA, PCR primers, and ELISA capture probe since *Salmonella* bacteria cultured from those samples were PCR-ELISA positive. The false negatives may be due to differences in sensitivity of the two methods, PCR-ELISA ($\geq 10^2$ CFU/ml) and culture (1 CFU/ml) (34). This difference in the sensitivity between PCR and culture may be due in part to volume constraints, since the sample volumes that can be processed by culture methods are larger than those for PCR (milliliters versus microliters) (32, 34, 48). However, an enrichment step could circumvent this problem by bringing salmonella cell numbers into the detection range of PCR-ELISA (34). In fact, we observed a lower percentage of false positives for samples following the enrichment step in TBG (8.3 versus 1.7%). Other possible explanations for the failure of PCR-ELISA include the degradation of *Salmonella* DNA before template was extracted or the presence of PCR inhibitors, despite the efforts that had been made to remove these inhibitors with the DNA isolation and purification kit (32, 56). The false positives that we observed for the *Salmonella*-specific PCR-ELISA may reflect the existence of sublethally damaged *Salmonella* cells in the samples, which may be nonculturable on the medium or under the culture conditions used in this study, or the presence of salmonellae in the sample at levels below PCR-ELISA detection limit (52). A preenrichment step may be required to allow damaged cells time to recover (34). The discrepancies between PCR and culture results may also be attributed to differences in enrichment, differential-selective media, and culture conditions in isolating certain *S. enterica* serotypes (52) as well as the failure to recognize atypical *Salmonella* colony types on differential-selective agars (21, 52).

Unlike *Salmonella*, we were unable to apply the statistical comparison of culture with *Campylobacter*-specific PCR-ELISA due to the organism's prevalence in the samples tested. One can sometimes find a flock of commercial birds in house that are campylobacter negative by culture (53). However, these flocks are hard to find, especially at certain times of year. When these flocks are caught caged and transported in soiled coops, they can become exposed to *Campylobacter* (51). They can also become exposed at the plant during hanging and picking (9). It would be difficult, if not impossible, to be sure that a flock that tested negative on the commercial farm remains negative during processing. With growing evidence that *Campylobacter* is transmitted vertically from the breeder parents to broiler progeny, it will be equally difficult to experimentally raise campylobacter-free chickens for the purposes of validating this PCR-ELISA (16, 28, 44). We were therefore unable to determine if PCR-ELISA was comparable to culture due to the absence of campylobacter-free samples. In the future, statistical validation of this PCR-ELISA will require testing of samples in which there is an expectation that a certain number of the sample(s) will be *Campylobacter* free (31, 38).

The PCR-ELISA detection scheme in our study could be a valuable tool in screening large numbers of samples for important food-borne pathogens like *Salmonella* and *Campylobacter*. It is rapid and cost-effective, taking 7 h for performance of the PCR-ELISA with a cost of about $3/sample, starting from the DNA extraction. When *Campylobacter* and *Salmonella* were screened for, few false negatives were found by PCR-ELISA, and that therefore allows us to focus culture on PCR-positive samples. Another advantage of PCR-ELISA is its 96-well microplate format, which allows larger sample sizes to be analyzed at the same time and makes automation possible.

PCR-ELISA has been recently used to quantify specific pathogens from clinical samples (1, 6, 17). This method is easier than competitive PCR methods developed for detection and quantification of specific DNA targets since it allows rapid detection of amplicons without the need of gel electrophoresis (21, 35). With competitive PCR, the quantity of target template is achieved by initially determining a standard curve in which various known amounts of target DNA are coamplified with a fixed amount of internal standard, and the ratio of targets and insertion sequence amplicons are plotted against the input amount of target DNA. Specific target DNA in clinical samples can be quantified through interpolation from the standard curve following coamplification with the same amount of internal standard as that used to construct the standard curve. However, the reliance on gel electrophoresis and cumbersome quantification systems has impeded quantitative PCR as a diagnostic assay (6). In this study, we constructed a standard curve by plotting OD values against *Campylobacter* cell count for different PCR cycles. The log-linear phase of the curve can be used in a semiquantification of bacterial cell concentration (CFU per milliliter) in a sample by knowing its relevant OD in PCR-ELISA (22). This PCR-ELISA detection system can be further extended to perform quantitative analysis of bacterial contamination by including a competitive internal standard in the PCR. Serial control DNA dilutions can be coamplified with a fixed number of internal standards in separate experiments, and amplicons can be detected by PCR-ELISA. The standard curve can be constructed by plotting the mean OD ratios of control DNA to internal standard amplicons against the number of copies of serially diluted control DNA (6). Quantitative PCR-ELISA can be a

potentially rapid detection method for the poultry industry. It may help in monitoring the contamination levels in processing plants and evaluating the performance of the hazard analysis and critical control point system. Quantitative data also facilitate setting up dose-response models in microbial risk assessment by which we can evaluate the impact of food-borne pathogens on human health (14, 26). However, the limitation of this PCR test as well as any PCR-based assay is its inherent inability to distinguish live from dead bacterial cells. Due to the short-half life of mRNA in the bacterial cell, RNA may serve as a better target template for PCR in development of rapid methods for detection and enumeration of live salmonellae or campylobacters on food products by PCR-ELISA (47, 55).

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